



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<p><b>(21) International Application Number:</b> PCT/US97/03217</p> <p><b>(22) International Filing Date:</b> 28 February 1997 (28.02.97)</p> <p><b>(30) Priority Data:</b>          60/012,679      1 March 1996 (01.03.96)      US</p> <p><b>(71) Applicants (for all designated States except US):</b> THE PROCTER &amp; GAMBLE COMPANY [US/US]; One Procter &amp; Gamble Plaza, Cincinnati, OH 45202 (US). CASE WESTERN RESERVE UNIVERSITY [US/US]; School of Medicine, 10900 Euclid Avenue, Cleveland, OH 44106-4946 (US).</p> <p><b>(72) Inventors; and</b>  <b>(75) Inventors/Applicants (for US only):</b> TINDAL, Michael, Howard [US/US]; 620 Cody Pass, Wyoming, OH 45215 (US). HAQQI, Tariq [IN/US]; Biomed Residence Building, 2109 Adelbert Road, Cleveland, OH 44106-4946 (US).</p> <p><b>(74) Agents:</b> REED, T., David et al.; The Procter &amp; Gamble Company, 5299 Spring Grove Avenue, Cincinnati, OH 45217 (US).</p>		<p><b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).</p> <p><b>Published</b>  <i>With international search report.          Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i></p>
<p><b>(54) Title:</b> A NOVEL DISINTEGRIN METALLOPROTEASE AND METHODS OF USE</p> <p><b>(57) Abstract</b></p> <p>This invention provides a method for identifying compounds capable of binding to the disintegrin protein, and determining the amount and affinity of a compound capable of binding to the disintegrin protein in a sample. This invention also provides a host cell comprising a recombinant expression vector to the disintegrin protein and a recombinant expression vector encoding to the disintegrin protein and the human disintegrin metalloprotease protein, fragment or mutant thereof, useful for these purposes. This invention also provides an <i>in vivo</i> or <i>in vitro</i> method for screening for osteoarthritis and other metalloprotease based diseases, capable of manufacture and use in a kit form.</p>		

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## A NOVEL DISINTEGRIN METALLOPROTEASE AND METHODS OF USE

### Field of the invention

The invention relates to a novel protein, its fragments and mutants and to its use in detecting and testing drugs for ailments, including osteoarthritis and others characterized by up regulation of metalloproteases.

### Background

A number of enzymes effect the breakdown of structural proteins and are structurally related metalloproteases. These include human skin fibroblast collagenase, human skin fibroblast gelatinase, human sputum collagenase and gelatinase, and human stromelysin. These are zinc-containing metalloprotease enzymes, as are the angiotensin-converting enzymes and the enkephalinases. Collagenase, stromelysin and related enzymes are important in mediating the symptomatology of a number of diseases, including rheumatoid arthritis (Mullins, D. E., et al., *Biochim Biophys Acta* (1983) 695:117-214); osteoarthritis (Henderson, B., et al., *Drugs of the Future* (1990) 15:495-508); the metastasis of tumor cells (ibid, Broadhurst, M. J., et al., *European Patent Application* 276,436 (published 1987), Reich, R., et al., 48 *Cancer Res* 3307-3312 (1988); and various ulcerated conditions. Ulcerative conditions can result in the cornea as the result of alkali burns or as a result of infection by *Pseudomonas aeruginosa*, *Acanthamoeba*, *Herpes simplex* and *vaccinia* viruses.

Other conditions characterized by undesired metalloprotease activity include periodontal disease, epidermolysis bullosa and scleritis. In view of the involvement of metalloproteases in a number of disease conditions, attempts have been made to prepare inhibitors to these enzymes. A number of such inhibitors are disclosed in the literature. The invention seeks to provide novel inhibitors, preferably specific to this protease, that have enhanced activity in treating diseases mediated or modulated by this protease.

Inhibitors of metalloproteases are useful in treating diseases caused, at least in part, by breakdown of structural proteins. A variety of inhibitors have been prepared, but there is a continuing need for metalloprotease inhibitor screens to design drugs for treating such diseases.

Metalloproteases are a broad class of proteins which have widely varied functions. Disintegrins are zinc metalloproteases, abundant in snake venom. Alternate cloning strategies could be used. Mammalian disintegrins are a family of proteins with about 18

known subgroups. They act as cell adhesion disrupters and are also known to be active in reproduction (for example, in fertilization of the egg by the sperm, including fusion thereof, and in sperm maturation).

These proteases and many others are uncovered in molecular biology and biochemistry. As a result, Genbank, a repository for gene sequences, provides several sequences of metalloproteases, including some said to encode fragments of disintegrins. For example, GenBank accession # Z48444 dated February 25, 1994 discloses 2407 bases of a rat gene said to be a rat disintegrin metalloprotease gene; GenBank accession # Z48579 dated March 2, 1995 discloses 1824 bases of a partial sequence of a gene said to be a human disintegrin metalloprotease gene; GenBank accession # Z21961 dated October 25, 1994, discloses 2397 bases of a partial sequence of a gene said to be a bovine zinc metalloprotease gene.

It would be advantageous to implicate metalloproteases in specific disease states, and to use these metalloproteases as tools to detect and ultimately cure, control or design cures for such diseases.

#### OBJECTS OF THE INVENTION

It is an object of the present invention to provide a method for identifying compounds capable of binding to the disintegrin protein.

It is also an object of the present invention to provide a host cell comprising a recombinant expression vector to the disintegrin protein and a recombinant expression vector encoding to the disintegrin protein.

It is also an object of the present invention to provide a method for screening for metalloprotease mediated diseases such as cancer, arthropathies (including ankylosing spondylitis, rheumatoid arthritis, gouty arthritis (gout), inflammatory arthritis, Lyme disease and osteoarthritis).

It is also an object of the present invention to provide an antibody to the protein useful in the screen, in the isolation of the protein or as a targeting moiety for the protein.

#### SUMMARY OF THE INVENTION

This invention provides a method for identifying compounds capable of binding to the disintegrin protein, and determining the amount and affinity of a compound capable of binding to the disintegrin protein in a sample.

This invention also provides a host cell comprising a recombinant expression vector to the disintegrin protein and a recombinant expression vector encoding to the disintegrin protein and the human disintegrin metalloprotease protein, fragment or mutant thereof, useful for these purposes.

This invention also provides an in vivo or in vitro method for screening for osteoarthritis and other metalloprotease based diseases, such as cancer, capable of manufacture and use in a kit form.

### DETAILED DESCRIPTION

As used herein, the terms "protein," "protease," and "metalloprotease" refer to a disintegrin. Preferably this is a human disintegrin as described below.

The term "antibody" refers to an antibody to a disintegrin, or fragment thereof. These may be monoclonal or polyclonal, and can be from any of several sources. The invention also contemplates fragments of these antibodies made by any method in the protein or peptide art.

The term "disease screen" refers to a screen for a disease or disease state. A disease state is the physiological or cellular or biochemical manifestation of the disease. Preferably this screen is used on body tissues or fluids of an animal or cell culture, using standard techniques, such as ELISA. It also contemplates "mapping" of disease in a whole body, such as by labeled antibody as described above given systemically: regardless of the detection method, preferable such detection methods include fluorescence, X-ray (including CAT scan), NMR (Including MRI), and the like.

The term "compound screen" is related to the methods and screens related to finding compounds, determining their affinity for the protease, or designing or selecting compounds based on the screen. In another embodiment, it contemplates the use of the three dimensional structure for drug design, preferable "rational drug design", as understood by the art. It may be preferred that the protease is in "essentially pure form", which refers to a protein reasonably free of other impurities, so as to make it useful for experiments or characterization. Use of this screening method assists the skilled artisan in finding novel structures, whether made by the chemist or by nature, which bind to and preferably inhibit the protease. These "inhibitors" may be useful in regulating or modulating the activity of the protease, and may be used to thus modulate the biological cascade that they function in. This approach affords new pharmaceutically useful compounds.

The term "disintegrin" refers to a disintegrin, a fragment thereof, a mutant thereof or a homologue which still retains its function. This term contemplates aggracanase, and other proteases which are involved in or modulate tissue remodelling. This contemplates disintegrins from differing species, and those prepared by recombinant methods, in vitro methods, or standard peptide synthesis. Preferably the protein is a human disintegrin or mutant thereof. For the purposes of defining the mutants of the protein the preferred

"native" protein is described in GenBank accession # Z48579, incorporated herein by reference and referred to in the sequence below. SEQ ID NO 1 describes a fragment of that DNA sequence and its transcript and SEQ ID NO 2 describes the coding region of the gene and its transcript. Homologue disintegrins include whole proteins with at least 90% homology as understood by the art, or fragments thereof. For example, a rat protein which is 95% homologous to that of SEQ ID NO. 1 or SEQ ID NO. 2 based on the peptide sequence derived from the DNA or cDNA sequence, and a bovine protein (similarly derived) being 97-98% homologous, are both considered homologues. Thus homologous cDNAs cloned from other organisms give rise to homologous proteins.

Likewise proteins may be considered homologues based on the amino acid sequence alone. Practical limitations of amino acid sequencing would allow one to determine that a protein is homologous to another using for example comparison of the first 50 amino acids of the protein. Hence 90% homology in would allow for 5 differing amino acids in the chain of the first 50 amino acids of the homologous protein.

The skilled artisan will appreciate that the degeneracy of the genetic code provides for differing DNA sequences to provide the same transcript, and thus the same peptide. In certain cases preparing the DNA sequence, which encodes for the same peptide, but differs from the native DNA include;

- ease of sequencing or synthesis;
- increased expression of the peptide; and
- preference of certain heterologous hosts for certain codons over others.

These practical considerations are widely known and provide embodiments that may be advantageous to the user of the invention. Thus it is clearly contemplated that the native DNA is not the only embodiment envisioned in this invention.

In addition it is apparent to the skilled artisan that fragments of the protein may be used in screening, drug design and the like, and that the entire peptide may not be required for the purposes of using the invention. Thus it is clearly contemplated that the skilled artisan will understand that the disclosure of the peptide and its uses contemplates the useful peptide fragments.

The practical considerations of protein expression, purification yield, stability, solubility, and the like, are considered by the skilled artisan when choosing whether to use a fragment, and the fragment to be used. As a result, using routine practices in the art, the artisan can, given this disclosure practice the invention using fragments of the protein as well.

The protein or protease itself can be used to determine the binding activity of small molecules to the protein. Drug screening using enzymatic targets is used in the art and can be employed using automated, high throughput technologies.

The inhibition of disintegrin activity may be a predictor of efficacy in the treatment of osteoarthritis, and other diseases involving degeneration of articular cartilage.

#### Gene therapy

Without being bound by theory it is thought that the metalloprotease is up regulated during osteoarthritis in tissues. We have surprisingly found that a human disintegrin is up-regulated in human chondrocytes during osteoarthritic conditions. Inhibition of signal transduction mechanism is efficacious in disrupting the cascade of events in osteoarthritis and other diseases involving cartilage degeneration. The skilled artisan will recognize that if up-regulation is a cause of the onset of arthritis, then interfering with the activity of this gene may be useful in treating osteoarthritis.

This is done by any of several methods, including gene (i.e., antisense) therapy.

#### Inhibitors of the disintegrin

The protease of the invention can be used to find inhibitors of the protease. Hence it is useful as a screening tool or for rational drug design. Without being bound by theory, the protease may modulate cellular remodeling and in fact may enhance extracellular matrix remodeling and thus enhance tissue breakdown. Hence inhibition of disintegrin provides a therapeutic route for treatment of diseases characterized by these processes.

In screening, a drug compound can be used to determine both the quality and quantity of inhibition. As a result such screening provides information for selection of actives, preferably small molecule actives, which are useful in treating these diseases.

In therapy, inhibition of disintegrin metalloprotease activity via binding of small molecular weight, synthetic metalloprotease inhibitors, such as those used to inhibit the matrix metalloproteases would be used to inhibit extracellular matrix remodeling.

#### Antibodies to the protein

Metalloproteases active at a particularly undesired location (e.g., an organ or certain types of cells) can be targeted by conjugating a metalloprotease inhibitor to a to an antibody or fragment thereof. Conjugation methods are known in the art.

The antibody of the invention can also be conjugated to solid supports. These conjugates can be used as affinity reagents for the purification of a desired metalloprotease, preferably a disintegrin.

In another aspect, the antibody of the invention is directly conjugated to a label. As the antibody binds to the metalloprotease, the label can be used to detect the presence of relatively high levels of metalloprotease in vivo or in vitro cell culture.

In addition, the metalloprotease inhibiting compounds can be conjugated to antibodies. Typical conjugation methods are known in the art. These antibodies are then useful both in therapy and in monitoring the dosage of the inhibitors.

For example, targeting ligand which specifically reacts with a marker for the intended target tissue can be used. Methods for coupling the invention compound to the targeting ligand are well known and are similar to those described below for coupling to carrier. The conjugates are formulated and administered as described above.

#### Preparation and Use of Antibodies:

Antibodies may be made by several methods, for example, the protein may be injected into suitable (e.g., mammalian) subjects including mice, rabbits, and the like. Preferred protocols involve repeated injection of the immunogen in the presence of adjuvants according to a schedule which boosts production of antibodies in the serum. The titers of the immune serum can readily be measured using immunoassay procedures, now standard in the art.

The antisera obtained can be used directly or monoclonal antibodies may be obtained by harvesting the peripheral blood lymphocytes or the spleen of the immunized animal and immortalizing the antibody-producing cells, followed by identifying the suitable antibody producers using standard immunoassay techniques.

Polyclonal or monoclonal preparations are useful in monitoring therapy or prophylaxis regimens involving the compounds of the invention. Suitable samples such as those derived from blood, serum, urine, or saliva can be tested for the presence of the protein at various times during the treatment protocol using standard immunoassay techniques which employ the antibody preparations of the invention.

These antibodies can also be coupled to labels such as scintigraphic labels, e.g., technetium 99 or I-131, using standard coupling methods. The labeled compounds are administered to subjects to determine the locations of excess amounts of one or more metalloproteases in vivo. Hence a labelled antibody to the protein would operate as a screening tool for such enhanced expression, indicating the disease.

The ability of the antibodies to bind metalloprotease selectively is thus taken advantage of to map the distribution of these enzymes in situ. The techniques can also be employed in histological procedures and the labeled antibodies can be used in competitive immunoassays.

Antibodies are advantageously coupled to other compounds or materials using known methods. For example, materials having a carboxyl functionality, the carboxyl residue can be reduced to an aldehyde and coupled to carrier through reaction with side chain amino groups, optionally followed by reduction of imino linkage formed. The carboxyl residue can also be reacted with side chain amino groups using condensing agents such as dicyclohexyl carbodiimide or other carbodiimide dehydrating agents. Linker compounds can also be used to effect the coupling; both homobifunctional and heterobifunctional linkers are available from Pierce Chemical Company, Rockford, Ill.

These antibodies, when conjugated to a suitable chromatography material are useful in isolating the protein. Separation methods using affinity chromatography are well known in the art, and are within the purview of the skilled artisan.

#### Disease marker

Without being bound by theory, expression of genes, and preferably this gene may have a restricted tissue distribution and its expression is up regulated by potential osteoarthritis mediators. Enhanced expression of this gene (and hence its protein) for example, in articular chondrocytes provides a marker to monitor the development, including the earliest, asymptomatic stages, and the progression of osteoarthritis. Hence an antibody raised to the protein would operate as a screening tool for such enhanced expression, indicating the disease.

In addition, when used in a disease screen, antibodies can be conjugated to chromophore or fluorophore containing materials, or can be conjugated to enzymes which produce chromophores or fluorophores in certain conditions. These conjugating materials and methods are well known in the art. When used in this manner detection of the protein by immunoassay is straightforward to the skilled artisan. Body fluids, for example can be screened in this manner for calibration, and detection of distribution of metalloproteases, or increased levels of these proteases.

When used in this way the invention is a useful diagnostic and/or clinical marker for metalloprotease mediated diseases, such as osteoarthritis or other articular cartilage degenerative diseases. When disease is detected, it may be treated before the onset of symptom or debilitation.

Furthermore, such antibodies can be used to target diseased tissue, for detection or treatment as described above.

#### EXAMPLES

The following non-limiting examples illustrate a preferred embodiment of the present invention, and briefly describe the uses of the present invention. These

examples are provided for the guidance of the skilled artisan, and do not limit the invention in any way. Armed with this disclosure and these examples the skilled artisan is capable of making and using the claimed invention.

Standard starting materials are used for these examples. Many of these materials are known and commercially available. For example *E. coli* CJ236 and JM101 are known strains, pUB110 is a known plasmid and Kunkel method mutagenesis is also well known in the art.

Variants may be made by expression systems and by various methods in various hosts, these methods are within the scope of the practice of the skilled artisan in molecular biology, biochemistry or other arts related to biotechnology.

#### Example 1

RNA was isolated from unstimulated and interleukin-1 stimulated cultures of normal human articular chondrocytes. The RNA was reverse transcribed into cDNA. The cDNA was subjected to a modified differential display procedure using a series of random primers.

PCR samples generated from both stimulated and unstimulated chondrocytes were electrophoreses in adjacent lanes on polyacrylamide gels. The differentially expressed band was excised from the gel, cloned, and sequenced. The differential expression of the gene was confirmed by RNase protection and nuclear run on experiments.

#### Example 2

A novel partial human cDNA coding the protein is cloned from primary cultures of interleukin-1 stimulated human articular (femoral head) chondrocytes, using known methods.

The same sequence is found, and the gene completed by screening of human cDNA libraries to obtain full length clones.

#### Example 3

The cloned DNA of example 2 was placed in pUB110 using known methods.

This plasmid is used to transform *E. coli* and provides a template for site-directed mutagenesis to create new mutants. Kunkel method mutagenesis performed altering GLN 1 ALA.

#### Example 4

[<sup>125</sup>I] disintegrin antibody is prepared using IODOBEADS (Pierce, Rockford, IL; immobilized chloramine-T on nonporous polystyrene beads). Lyophilized antibody (2 µg) is taken up in 50 µl of 10 mM acetic acid and added to 450 µl of phosphate-buffered saline (PBS) (Sigma, St. Louis, MO) on ice. To the tube is added 500 µCurie of <sup>125</sup>I

(Amersham, Arlington Heights, IL) (2200Ci/mmol) in 5  $\mu$ l, and one IODOBEAD. The reaction is incubated on ice for 10 min with occasional shaking. The reaction is then terminated by removal of the reaction from the IODOBEAD. To remove unreacted  $^{125}\text{I}$ , the mixture is applied to a PD-10 gel filtration column.

#### Example 5

A fluorogenic peptide (Bachem, Guelph Mills, King of Prussia, Pa) is mixed with the disintegrin and change in the fluorescence is evaluated at 2 min, as a control. Then the fluorogenic peptide is mixed with the disintegrin in the presence of the compound in evaluation in a separate run, with evaluation at 2 minutes. Data are evaluated using standard methodology to provide relative binding of the evaluated compound.

#### Example 6

0.5ml of synovial fluid from the left knee of a patient is withdrawn and tested for elevated levels disintegrin by ELISA. The results indicate higher than normal disintegrin level. The patient is prescribed a prophylactic dose of a disintegrin inhibitor, and is administered an injection of same in the left knee before leaving the clinician's office.

#### Example 7

Inhibition of extracellular matrix remodeling is explored via inhibition of disintegrin metalloprotease activity. Using a small molecular weight, synthetic metalloprotease inhibitor, such as those used to inhibit the matrix metalloproteases, tissue integrity and proteoglycan is monitored.

A sample of mouse derived articular cartilage is grown in a 1 micromolar solution of a small molecular weight disintegrin inhibitor. The experiment is controlled and compared to an identical culture grown with no inhibitor.

The assay of the culture after 7 days shows that the inhibited culture has less tissue breakdown and less proteoglycan present in the serum of the culture. The result is consistent with the inhibited aggrecanase activity. Inhibition of aggrecanase would inhibit tissue breakdown and reduce the release of proteoglycan.

#### Example 8

Inhibition of proteolytic processing resulting in the release from the membrane bound form of the disintegrin metalloprotease domain inhibits "second messenger" signaling of the membrane bound disintegrin molecule. Such second messenger signaling would result in cellular phenotypic changes, changes in gene expression, changes in mitotic activity, and the like.

Cells known to contain disintegrin are treated with a serine protease. Proteins released from the cell are measured by standard methods. Specifically the

metalloprotease activity is monitored via literature methods. The amount of metalloprotease released is correlated to the amount of serine protease used to treat the cells.

Increases, versus control, in src tyrosine kinase activity are measured by Western blot analysis of intracellular proteins using monoclonal antibodies specific for phosphotyrosine following cleavage and release of the disintegrin metalloprotease. Controls are cells that have not been treated with serine protease.

src tyrosine kinase activity in the cell (or in cell culture) is measured by literature methods. Release of the metalloprotease domain of the disintegrin is also monitored via literature methods. There is a direct correlation between release of the metalloprotease domain and increases in intracellular src tyrosine kinase activity. This result is consistent with stimulation of disintegrin-mediated cell signalling by stimulation of the src tyrosine kinase cascade.

#### Example 9

Inhibition of intercellular adhesion molecules, or extracellular matrix components results in the inhibition of phenotypic changes, including changes in cell shape, associated with such interactions, as described in Example 8.

Integrin binding is measured with a peptide containing the sequence RGD, using the protocol of Example 8. Integrin binding is measured via competitive assay, using cellular changes in shape visible via microscopy. The peptide inhibits the cellular changes as in Example 8.

This result is consistent with competition with or blocking of the interaction of disintegrin. The RGD peptide inhibits cellular changes in chondrocytes. The osteoarthritis phenotype, characterized by increased matrix synthesis and accelerated matrix metalloprotease activity does not occur. Other readily assayable cellular changes can be used to monitor this result, including gene expression, changes in mitotic activity, and the like.

#### Example 10

A small molecular weight metalloprotease inhibitor is used to treat a tissue culture according to the method of Example 7. The release of TNF- $\alpha$  from the cell membrane is measured by literature methods. The inhibitor of Example 7 also decreases the amount of TNF- $\alpha$  secreted from the cell membrane.

This is consistent with the theory that inhibition of disintegrin metalloprotease activity will result in the inhibition of a disintegrin associated inflammation cascade and

secretase activity. It is contemplated that monitoring the release of cytokines or IL-1 from the cell membrane, and the like will produce the same result.

All references described herein are hereby incorporated by reference.

While particular embodiments of the subject invention have been described, it will be obvious to those skilled in the art that various changes and modifications of the subject invention can be made without departing from the spirit and scope of the invention. It is intended to cover, in the appended claims, all such modifications that are within the scope of this invention.

## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

- (i) APPLICANT: TINDAL, MICHAEL H.  
HAQQI, TARIQ M.
- (ii) TITLE OF INVENTION: USE OF A NOVEL DISINTEGRIN  
METALLOPROTEASE, ITS MUTANTS, FRAGMENTS AND THE LIKE
- (iii) NUMBER OF SEQUENCES: 4
- (iv) CORRESPONDENCE ADDRESS:
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  - (C) CITY: MASON
  - (D) STATE: OHIO
  - (E) COUNTRY: USA
  - (F) ZIP: 45040-9462
- (v) COMPUTER READABLE FORM:
  - (A) MEDIUM TYPE: Floppy disk
  - (B) COMPUTER: IBM PC compatible
  - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
  - (D) SOFTWARE: PatentIn Release #1.0, Version #1.30
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  - (B) FILING DATE: 01-MAR-1996
  - (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
  - (A) NAME: HAKE, RICHARD A.
  - (B) REGISTRATION NUMBER: 37,343
  - (C) REFERENCE/DOCKET NUMBER: 5980
- (ix) TELECOMMUNICATION INFORMATION:
  - (A) TELEPHONE: 513/622-0087
  - (B) TELEFAX: 513/622-0270

## (2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 1961 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (ix) FEATURE:
  - (A) NAME/KEY: CDS
  - (B) LOCATION: 2..1474
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

C	CAG	ACC	ACA	GAC	TTC	TCC	GGA	ATC	CGT	AAC	ATC	AGT	TTC	ATG	GTG		46
	Gln	Thr	Thr	Asp	Phe	Ser	Gly	Ile	Arg	Asn	Ile	Ser	Phe	Met	Val		
	1				5					10					15		
AAA	CGC	ATA	AGA	ATC	AAT	ACA	ACT	GCT	GAT	GAG	AAG	GAC	CCT	ACA	AAT		94
Lys	Arg	Ile	Arg	Ile	Asn	Thr	Thr	Ala	Asp	Glu	Lys	Asp	Pro	Thr	Asn		
				20					25					30			
CCT	TTC	CGT	TTC	CCA	AAT	ATT	AGT	GTG	GAG	AAG	TTT	CTG	GAA	TTG	AAT		142
Pro	Phe	Arg	Phe	Pro	Asn	Ile	Ser	Val	Glu	Lys	Phe	Leu	Glu	Leu	Asn		
			35					40					45				
TCT	GAG	CAG	AAT	CAT	GAT	GAC	TAC	TGT	TTG	GCC	TAT	GTC	TTC	ACA	GAC		190
Ser	Glu	Gln	Asn	His	Asp	Asp	Tyr	Cys	Leu	Ala	Tyr	Val	Phe	Thr	Asp		
		50					55					60					
CGA	GAT	TTT	GAT	GAT	GGC	GTA	CTT	GGT	CTG	GCT	TGG	GTT	GGA	GCA	CCT		238
Arg	Asp	Phe	Asp	Asp	Gly	Val	Leu	Gly	Leu	Ala	Trp	Val	Gly	Ala	Pro		
	65					70					75						
TCA	GGA	AGC	TCT	GGA	GGA	ATA	TGT	GAA	AAA	AGT	AAA	CTC	TAT	TCA	GAT		286
Ser	Gly	Ser	Ser	Gly	Gly	Ile	Cys	Glu	Lys	Ser	Lys	Leu	Tyr	Ser	Asp		
	80				85					90					95		
GGT	AAG	AAG	AAG	TCC	TTA	AAC	ACT	GGA	ATT	ATT	ACT	GTT	CAG	AAC	TAT		334
Gly	Lys	Lys	Lys	Ser	Leu	Asn	Thr	Gly	Ile	Ile	Thr	Val	Gln	Asn	Tyr		
				100					105					110			
GGG	TCT	CAT	GTA	CCT	CCC	AAA	GTC	TCT	CAC	ATT	ACT	TTT	GCT	CAC	GAA		382
Gly	Ser	His	Val	Pro	Pro	Lys	Val	Ser	His	Ile	Thr	Phe	Ala	His	Glu		
			115					120					125				
GTT	GGA	CAT	AAC	TTT	GGA	TCC	CCA	CAT	GAT	TCT	GGA	ACA	GAG	TGC	ACA		430
Val	Gly	His	Asn	Phe	Gly	Ser	Pro	His	Asp	Ser	Gly	Thr	Glu	Cys	Thr		
			130				135					140					
CCA	GGA	GAA	TCT	AAG	AAT	TTG	GGT	CAA	AAA	GAA	AAT	GGC	AAT	TAC	ATC		478
Pro	Gly	Glu	Ser	Lys	Asn	Leu	Gly	Gln	Lys	Glu	Asn	Gly	Asn	Tyr	Ile		
	145					150					155						
ATG	TAT	GCA	AGA	GCA	ACA	TCT	GGG	GAC	AAA	CTT	AAC	AAC	AAT	AAA	TTC		526
Met	Tyr	Ala	Arg	Ala	Thr	Ser	Gly	Asp	Lys	Leu	Asn	Asn	Asn	Lys	Phe		
	160				165					170					175		
TCA	CTC	TGT	AGT	ATT	AGA	AAT	ATA	AGC	CAA	GTT	CTT	GAG	AAG	AAG	AGA		574
Ser	Leu	Cys	Ser	Ile	Arg	Asn	Ile	Ser	Gln	Val	Leu	Glu	Lys	Lys	Arg		
				180					185					190			
AAC	AAC	TGT	TTT	GTT	GAA	TCT	GGC	CAA	CCT	ATT	TGT	GGA	AAT	GGA	ATG		622
Asn	Asn	Cys	Phe	Val	Glu	Ser	Gly	Gln	Pro	Ile	Cys	Gly	Asn	Gly	Met		
			195					200					205				
GTA	GAA	CAA	GGT	GAA	GAA	TGT	GAT	TGT	GGC	TAT	AGT	GAC	CAG	TGT	AAA		670
Val	Glu	Gln	Gly	Glu	Glu	Cys	Asp	Cys	Gly	Tyr	Ser	Asp	Gln	Cys	Lys		
		210					215					220					
GAT	GAA	TGC	TGC	TTC	GAT	GCA	AAT	CAA	CCA	GAG	GGA	AGA	AAA	TGC	AAA		718
Asp	Glu	Cys	Cys	Phe	Asp	Ala	Asn	Gln	Pro	Glu	Gly	Arg	Lys	Cys	Lys		

225	230	235	
CTG AAA CCT GGG AAA CAG TGC AGT CCA AGT CAA GGT CCT TGT TGT ACA Leu Lys Pro Gly Lys Gln Cys Ser Pro Ser Gln Gly Pro Cys Cys Thr 240 245 250 255			766
GCA CAG TGT GCA TTC AAG TCA AAG TCT GAG AAG TGT CGG GAT GAT TCA Ala Gln Cys Ala Phe Lys Ser Lys Ser Glu Lys Cys Arg Asp Asp Ser 260 265 270			814
GAC TGT GCA AGG GAA GGA ATA TGT AAT GGC TTC ACA GCT CTC TGC CCA Asp Cys Ala Arg Glu Gly Ile Cys Asn Gly Phe Thr Ala Leu Cys Pro 275 280 285			862
GCA TCT GAC CCT AAA CCA AAC TTC ACA GAC TGT AAT AGG CAT ACA CAA Ala Ser Asp Pro Lys Pro Asn Phe Thr Asp Cys Asn Arg His Thr Gln 290 295 300			910
GTG TGC ATT AAT GGG CAA TGT GCA GGT TCT ATC TGT GAG AAA TAT GGC Val Cys Ile Asn Gly Gln Cys Ala Gly Ser Ile Cys Glu Lys Tyr Gly 305 310 315			958
TTA GAG GAG TGT ACG TGT GCC AGT TCT GAT GGC AAA GAT GAT AAA GAA Leu Glu Glu Cys Thr Cys Ala Ser Ser Asp Gly Lys Asp Asp Lys Glu 320 325 330 335			1006
TTA TGC CAT GTA TGC TGT ATG AAG AAA ATG GAC CCA TCA ACT TGT GCC Leu Cys His Val Cys Cys Met Lys Lys Met Asp Pro Ser Thr Cys Ala 340 345 350			1054
AGT ACA GGG TCT GTG CAG TGG AGT AGG CAC TTC AGT GGT CGA ACC ATC Ser Thr Gly Ser Val Gln Trp Ser Arg His Phe Ser Gly Arg Thr Ile 355 360 365			1102
ACC CTG CAA CCT GGA TCC CCT TGC AAC GAT TTT AGA GGT TAC TGT GAT Thr Leu Gln Pro Gly Ser Pro Cys Asn Asp Phe Arg Gly Tyr Cys Asp 370 375 380			1150
GTT TTC ATG CGG TGC AGA TTA GTA GAT GCT GAT GGT CCT CTA GCT AGG Val Phe Met Arg Cys Arg Leu Val Asp Ala Asp Gly Pro Leu Ala Arg 385 390 395			1198
CTT AAA AAA GCA ATT TTT AGT CCA GAG CTC TAT GAA AAC ATT GCT GAA Leu Lys Lys Ala Ile Phe Ser Pro Glu Leu Tyr Glu Asn Ile Ala Glu 400 405 410 415			1246
TGG ATT GTG GCT CAT TGG TGG GCA GTA TTA CTT ATG GGA ATT GCT CTG Trp Ile Val Ala His Trp Trp Ala Val Leu Leu Met Gly Ile Ala Leu 420 425 430			1294
ATC ATG CTA ATG GCT GGA TTT ATT AAG ATA TGC AGT GTT CAT ACT CCA Ile Met Leu Met Ala Gly Phe Ile Lys Ile Cys Ser Val His Thr Pro 435 440 445			1342
AGT AGT AAT CCA AAG TTG CCT CCT CCT AAA CCA CTT CCA GGC ACT TTA Ser Ser Asn Pro Lys Leu Pro Pro Pro Lys Pro Leu Pro Gly Thr Leu 450 455 460			1390
AAG AGG AGG AGA CCT CCA CAG CCC ATT CAG CAA CCC CAG CGT CAG CGG			1438

Lys	Arg	Arg	Arg	Pro	Pro	Gln	Pro	Ile	Gln	Gln	Pro	Gln	Arg	Gln	Arg		
465						470					475						
CCC	CGA	GAG	AGT	TAT	CAA	ATG	GGA	CAC	ATG	AGA	CGC	TA	ACTGCAGC				1484
Pro	Arg	Glu	Ser	Tyr	Gln	Met	Gly	His	Met	Arg	Arg						
480					485					490							
TTTTGCCTTG	GTTCTTCCTA	GTGCCTACAA	TGGGAAAAC	TCACTCCAAA	GAGAAACCTA												1544
TTAAGTCATC	ATCTCCAAAC	TAAACCCTCA	CAAGTAACAG	TTGAAGAAAA	AATGGCAAGA												1604
GATCATATCC	TCAGACCAGG	TGGAATTACT	TAAATTTTAA	AGCCTGAAAA	TTCCAATTTG												1664
GGGGTGGGAG	GTGGAAAAGG	AACCCAATTT	TCTTATGAAC	AGATATTTTT	AACTTAATGG												1724
CACAAAGTCT	TAGAATATTA	TTATGTGCCC	CGTGTTCCT	GTTCTTCGTT	GCTGCATTTT												1784
CTTCACTTGC	AGGCAAACCT	GGCTCTCAAT	AACTTTTTCG	GTCCAGACCA	CAGACTTCTC												1844
CGGAATCCGT	AACATCAGTT	TCATGGTGAA	ACGCATAAGA	ATCAATACAA	CTGCTGATGA												1904
GAAGGACCCT	ACAAATCCTT	TCCGTTTCCC	AAATATTAGT	GTGGAGAAGT	TAAACAA												1961

## (2) INFORMATION FOR SEQ ID NO:2:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 491 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Gln	Thr	Thr	Asp	Phe	Ser	Gly	Ile	Arg	Asn	Ile	Ser	Phe	Met	Val	Lys		
1				5					10					15			
Arg	Ile	Arg	Ile	Asn	Thr	Thr	Ala	Asp	Glu	Lys	Asp	Pro	Thr	Asn	Pro		
			20					25					30				
Phe	Arg	Phe	Pro	Asn	Ile	Ser	Val	Glu	Lys	Phe	Leu	Glu	Leu	Asn	Ser		
		35					40					45					
Glu	Gln	Asn	His	Asp	Asp	Tyr	Cys	Leu	Ala	Tyr	Val	Phe	Thr	Asp	Arg		
50					55						60						
Asp	Phe	Asp	Asp	Gly	Val	Leu	Gly	Leu	Ala	Trp	Val	Gly	Ala	Pro	Ser		
65					70				75						80		
Gly	Ser	Ser	Gly	Gly	Ile	Cys	Glu	Lys	Ser	Lys	Leu	Tyr	Ser	Asp	Gly		
			85						90					95			
Lys	Lys	Lys	Ser	Leu	Asn	Thr	Gly	Ile	Ile	Thr	Val	Gln	Asn	Tyr	Gly		
			100					105					110				
Ser	His	Val	Pro	Pro	Lys	Val	Ser	His	Ile	Thr	Phe	Ala	His	Glu	Val		
		115					120					125					

Gly His Asn Phe Gly Ser Pro His Asp Ser Gly Thr Glu Cys Thr Pro  
 130 135 140  
 Gly Glu Ser Lys Asn Leu Gly Gln Lys Glu Asn Gly Asn Tyr Ile Met  
 145 150 155 160  
 Tyr Ala Arg Ala Thr Ser Gly Asp Lys Leu Asn Asn Asn Lys Phe Ser  
 165 170 175  
 Leu Cys Ser Ile Arg Asn Ile Ser Gln Val Leu Glu Lys Lys Arg Asn  
 180 185 190  
 Asn Cys Phe Val Glu Ser Gly Gln Pro Ile Cys Gly Asn Gly Met Val  
 195 200 205  
 Glu Gln Gly Glu Glu Cys Asp Cys Gly Tyr Ser Asp Gln Cys Lys Asp  
 210 215 220  
 Glu Cys Cys Phe Asp Ala Asn Gln Pro Glu Gly Arg Lys Cys Lys Leu  
 225 230 235 240  
 Lys Pro Gly Lys Gln Cys Ser Pro Ser Gln Gly Pro Cys Cys Thr Ala  
 245 250 255  
 Gln Cys Ala Phe Lys Ser Lys Ser Glu Lys Cys Arg Asp Asp Ser Asp  
 260 265 270  
 Cys Ala Arg Glu Gly Ile Cys Asn Gly Phe Thr Ala Leu Cys Pro Ala  
 275 280 285  
 Ser Asp Pro Lys Pro Asn Phe Thr Asp Cys Asn Arg His Thr Gln Val  
 290 295 300  
 Cys Ile Asn Gly Gln Cys Ala Gly Ser Ile Cys Glu Lys Tyr Gly Leu  
 305 310 315 320  
 Glu Glu Cys Thr Cys Ala Ser Ser Asp Gly Lys Asp Asp Lys Glu Leu  
 325 330 335  
 Cys His Val Cys Cys Met Lys Lys Met Asp Pro Ser Thr Cys Ala Ser  
 340 345 350  
 Thr Gly Ser Val Gln Trp Ser Arg His Phe Ser Gly Arg Thr Ile Thr  
 355 360 365  
 Leu Gln Pro Gly Ser Pro Cys Asn Asp Phe Arg Gly Tyr Cys Asp Val  
 370 375 380  
 Phe Met Arg Cys Arg Leu Val Asp Ala Asp Gly Pro Leu Ala Arg Leu  
 385 390 395 400  
 Lys Lys Ala Ile Phe Ser Pro Glu Leu Tyr Glu Asn Ile Ala Glu Trp  
 405 410 415  
 Ile Val Ala His Trp Trp Ala Val Leu Leu Met Gly Ile Ala Leu Ile  
 420 425 430  
 Met Leu Met Ala Gly Phe Ile Lys Ile Cys Ser Val His Thr Pro Ser  
 435 440 445

Ser Asn Pro Lys Leu Pro Pro Pro Lys Pro Leu Pro Gly Thr Leu Lys  
 450 455 460

Arg Arg Arg Pro Pro Gln Pro Ile Gln Gln Pro Gln Arg Gln Arg Pro  
 465 470 475 480

Arg Glu Ser Tyr Gln Met Gly His Met Arg Arg  
 485 490

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2763 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 17..2414

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GGCGGCGGCA CGGAAG ATG GTG TTG CTG AGA GTG TTA ATT CTG CTC CTC	49
Met Val Leu Leu Arg Val Leu Ile Leu Leu Leu	
495 500	
TCC TGG GCG GCG GGG ATG GGA GGT CAG TAT GGG AAT CCT TTA AAT AAA	97
Ser Trp Ala Ala Gly Met Gly Gly Gln Tyr Gly Asn Pro Leu Asn Lys	
505 510 515	
TAT ATC AGA CAT TAT GAA GGA TTA TCT TAC AAT GTG GAT TCA TTA CAC	145
Tyr Ile Arg His Tyr Glu Gly Leu Ser Tyr Asn Val Asp Ser Leu His	
520 525 530	
CAA AAA CAC CAG CGT GCC AAA AGA GCA GTC TCA CAT GAA GAC CAA TTT	193
Gln Lys His Gln Arg Ala Lys Arg Ala Val Ser His Glu Asp Gln Phe	
535 540 545 550	
TTA CGT CTA GAT TTC CAT GCC CAT GGA AGA CAT TTC AAC CTA CGA ATG	241
Leu Arg Leu Asp Phe His Ala His Gly Arg His Phe Asn Leu Arg Met	
555 560 565	
AAG AGG GAC ACT TCC CTT TTC AGT GAT GAA TTT AAA GTA GAA ACA TCA	289
Lys Arg Asp Thr Ser Leu Phe Ser Asp Glu Phe Lys Val Glu Thr Ser	
570 575 580	
AAT AAA GTA CTT GAT TAT GAT ACC TCT CAT ATT TAC ACT GGA CAT ATT	337
Asn Lys Val Leu Asp Tyr Asp Thr Ser His Ile Tyr Thr Gly His Ile	
585 590 595	
TAT GGT GAA GAA GGA AGT TTT AGC CAT GGG TCT GTT ATT GAT GGA AGA	385
Tyr Gly Glu Glu Gly Ser Phe Ser His Gly Ser Val Ile Asp Gly Arg	
600 605 610	

TTT GAA GGA TTC ATC CAG ACT CGT GGT GGC ACA TTT TAT GTT GAG CCA Phe Glu Gly Phe Ile Gln Thr Arg Gly Gly Thr Phe Tyr Val Glu Pro 615 620 625 630	433
GCA GAG AGA TAT ATT AAA GAC CGA ACT CTG CCA TTT CAC TCT GTC ATT Ala Glu Arg Tyr Ile Lys Asp Arg Thr Leu Pro Phe His Ser Val Ile 635 640 645	481
TAT CAT GAA GAT GAT ATT AGT GAA AGG CTT AAA CTG AGG CTT AGA AAA Tyr His Glu Asp Asp Ile Ser Glu Arg Leu Lys Leu Arg Leu Arg Lys 650 655 660	529
CTT ATG TCA CTT GAG TTG TGG ACC TCC TGT TGT TTA CCC TGT GCT CTT Leu Met Ser Leu Glu Leu Trp Thr Ser Cys Cys Leu Pro Cys Ala Leu 665 670 675	577
CTG CTT CAC TCA TGG AAG AAA GCT GTA AAT TCT CAC TGC CTT TAC TTC Leu Leu His Ser Trp Lys Lys Ala Val Asn Ser His Cys Leu Tyr Phe 680 685 690	625
AAG GAT TTC TGG GGC TTT TCT GAA ATC TAC TAT CCC CAT AAA TAC GGT Lys Asp Phe Trp Gly Phe Ser Glu Ile Tyr Tyr Pro His Lys Tyr Gly 695 700 705 710	673
CCT CAG GGC GGC TGT GCA GAT CAT TCA GTA TTT GAA AGA ATG AGG AAA Pro Gln Gly Gly Cys Ala Asp His Ser Val Phe Glu Arg Met Arg Lys 715 720 725	721
TAC CAG ATG ACT GGT GTA GAG GAA GTA ACA CAG ATA CCT CAA GAA GAA Tyr Gln Met Thr Gly Val Glu Glu Val Thr Gln Ile Pro Gln Glu Glu 730 735 740	769
CAT GCT GCT AAT GGT CCA GAA CTT CTG AGG AAA AGA CGT ACA ACT TCA His Ala Ala Asn Gly Pro Glu Leu Leu Arg Lys Arg Arg Thr Thr Ser 745 750 755	817
GCT GAA AAA AAT ACT TGT CAG CTT TAT ATT CAG ACT GAT CAT TTG TTC Ala Glu Lys Asn Thr Cys Gln Leu Tyr Ile Gln Thr Asp His Leu Phe 760 765 770	865
TTT AAA TAT TAC GGA ACA CGA GAA GCT GTG ATT GCC CAG ATA TCC AGT Phe Lys Tyr Tyr Gly Thr Arg Glu Ala Val Ile Ala Gln Ile Ser Ser 775 780 785 790	913
CAT GTT AAA GCG ATT GAT ACA ATT TAC CAG ACC ACA GAC TTC TCC GGA His Val Lys Ala Ile Asp Thr Ile Tyr Gln Thr Thr Asp Phe Ser Gly 795 800 805	961
ATC CGT AAC ATC AGT TTC ATG GTG AAA CGC ATA AGA ATC AAT ACA ACT Ile Arg Asn Ile Ser Phe Met Val Lys Arg Ile Arg Ile Asn Thr Thr 810 815 820	1009
GCT GAT GAG AAG GAC CCT ACA AAT CCT TTC CGT TTC CCA AAT ATT AGT Ala Asp Glu Lys Asp Pro Thr Asn Pro Phe Arg Phe Pro Asn Ile Ser 825 830 835	1057
GTG GAG AAG TTT CTG GAA TTG AAT TCT GAG CAG AAT CAT GAT GAC TAC Val Glu Lys Phe Leu Glu Leu Asn Ser Glu Gln Asn His Asp Asp Tyr	1105

840	845	850	
TGT TTG GCC TAT GTC TTC ACA GAC CGA GAT TTT GAT GAT GGC GTA CTT Cys Leu Ala Tyr Val Phe Thr Asp Arg Asp Phe Asp Asp Gly Val Leu 855 860 865 870	1153		
GGT CTG GCT TGG GTT GGA GCA CCT TCA GGA AGC TCT GGA GGA ATA TGT Gly Leu Ala Trp Val Gly Ala Pro Ser Gly Ser Ser Gly Gly Ile Cys 875 880 885	1201		
GAA AAA AGT AAA CTC TAT TCA GAT GGT AAG AAG AAG TCC TTA AAC ACT Glu Lys Ser Lys Leu Tyr Ser Asp Gly Lys Lys Lys Ser Leu Asn Thr 890 895 900	1249		
GGA ATT ATT ACT GTT CAG AAC TAT GGG TCT CAT GTA CCT CCC AAA GTC Gly Ile Ile Thr Val Gln Asn Tyr Gly Ser His Val Pro Pro Lys Val 905 910 915	1297		
TCT CAC ATT ACT TTT GCT CAC GAA GTT GGA CAT AAC TTT GGA TCC CCA Ser His Ile Thr Phe Ala His Glu Val Gly His Asn Phe Gly Ser Pro 920 925 930	1345		
CAT GAT TCT GGA ACA GAG TGC ACA CCA GGA GAA TCT AAG AAT TTG GGT His Asp Ser Gly Thr Glu Cys Thr Pro Gly Glu Ser Lys Asn Leu Gly 935 940 945 950	1393		
CAA AAA GAA AAT GGC AAT TAC ATC ATG TAT GCA AGA GCA ACA TCT GGG Gln Lys Glu Asn Gly Asn Tyr Ile Met Tyr Ala Arg Ala Thr Ser Gly 955 960 965	1441		
GAC AAA CTT AAC AAC AAT AAA TTC TCA CTC TGT AGT ATT AGA AAT ATA Asp Lys Leu Asn Asn Asn Lys Phe Ser Leu Cys Ser Ile Arg Asn Ile 970 975 980	1489		
AGC CAA GTT CTT GAG AAG AAG AGA AAC AAC TGT TTT GTT GAA TCT GGC Ser Gln Val Leu Glu Lys Lys Arg Asn Asn Cys Phe Val Glu Ser Gly 985 990 995	1537		
CAA CCT ATT TGT GGA AAT GGA ATG GTA GAA CAA GGT GAA GAA TGT GAT Gln Pro Ile Cys Gly Asn Gly Met Val Glu Gln Gly Glu Glu Cys Asp 1000 1005 1010	1585		
TGT GGC TAT AGT GAC CAG TGT AAA GAT GAA TGC TGC TTC GAT GCA AAT Cys Gly Tyr Ser Asp Gln Cys Lys Asp Glu Cys Cys Phe Asp Ala Asn 1015 1020 1025 1030	1633		
CAA CCA GAG GGA AGA AAA TGC AAA CTG AAA CCT GGG AAA CAG TGC AGT Gln Pro Glu Gly Arg Lys Cys Lys Leu Lys Pro Gly Lys Gln Cys Ser 1035 1040 1045	1681		
CCA AGT CAA GGT CCT TGT TGT ACA GCA CAG TGT GCA TTC AAG TCA AAG Pro Ser Gln Gly Pro Cys Cys Thr Ala Gln Cys Ala Phe Lys Ser Lys 1050 1055 1060	1729		
TCT GAG AAG TGT CGG GAT GAT TCA GAC TGT GCA AGG GAA GGA ATA TGT Ser Glu Lys Cys Arg Asp Asp Ser Asp Cys Ala Arg Glu Gly Ile Cys 1065 1070 1075	1777		
AAT GGC TTC ACA GCT CTC TGC CCA GCA TCT GAC CCT AAA CCA AAC TTC	1825		

Asn Gly Phe Thr Ala Leu Cys Pro Ala Ser Asp Pro Lys Pro Asn Phe 1080 1085 1090	
ACA GAC TGT AAT AGG CAT ACA CAA GTG TGC ATT AAT GGG CAA TGT GCA Thr Asp Cys Asn Arg His Thr Gln Val Cys Ile Asn Gly Gln Cys Ala 1095 1100 1105 1110	1873
GGT TCT ATC TGT GAG AAA TAT GGC TTA GAG GAG TGT ACG TGT GCC AGT Gly Ser Ile Cys Glu Lys Tyr Gly Leu Glu Glu Cys Thr Cys Ala Ser 1115 1120 1125	1921
TCT GAT GGC AAA GAT GAT AAA GAA TTA TGC CAT GTA TGC TGT ATG AAG Ser Asp Gly Lys Asp Asp Lys Glu Leu Cys His Val Cys Cys Met Lys 1130 1135 1140	1969
AAA ATG GAC CCA TCA ACT TGT GCC AGT ACA GGG TCT GTG CAG TGG AGT Lys Met Asp Pro Ser Thr Cys Ala Ser Thr Gly Ser Val Gln Trp Ser 1145 1150 1155	2017
AGG CAC TTC AGT GGT CGA ACC ATC ACC CTG CAA CCT GGA TCC CCT TGC Arg His Phe Ser Gly Arg Thr Ile Thr Leu Gln Pro Gly Ser Pro Cys 1160 1165 1170	2065
AAC GAT TTT AGA GGT TAC TGT GAT GTT TTC ATG CGG TGC AGA TTA GTA Asn Asp Phe Arg Gly Tyr Cys Asp Val Phe Met Arg Cys Arg Leu Val 1175 1180 1185 1190	2113
GAT GCT GAT GGT CCT CTA GCT AGG CTT AAA AAA GCA ATT TTT AGT CCA Asp Ala Asp Gly Pro Leu Ala Arg Leu Lys Lys Ala Ile Phe Ser Pro 1195 1200 1205	2161
GAG CTC TAT GAA AAC ATT GCT GAA TGG ATT GTG GCT CAT TGG TGG GCA Glu Leu Tyr Glu Asn Ile Ala Glu Trp Ile Val Ala His Trp Trp Ala 1210 1215 1220	2209
GTA TTA CTT ATG GGA ATT GCT CTG ATC ATG CTA ATG GCT GGA TTT ATT Val Leu Leu Met Gly Ile Ala Leu Ile Met Leu Met Ala Gly Phe Ile 1225 1230 1235	2257
AAG ATA TGC AGT GTT CAT ACT CCA AGT AGT AAT CCA AAG TTG CCT CCT Lys Ile Cys Ser Val His Thr Pro Ser Ser Asn Pro Lys Leu Pro Pro 1240 1245 1250	2305
CCT AAA CCA CTT CCA GGC ACT TTA AAG AGG AGG AGA CCT CCA CAG CCC Pro Lys Pro Leu Pro Gly Thr Leu Lys Arg Arg Arg Pro Pro Gln Pro 1255 1260 1265 1270	2353
ATT CAG CAA CCC CAG CGT CAG CGG CCC CGA GAG AGT TAT CAA ATG GGA Ile Gln Gln Pro Gln Arg Gln Arg Pro Arg Glu Ser Tyr Gln Met Gly 1275 1280 1285	2401
CAC ATG AGA CGC T AACTGCAGCT TTTGCCCTTG TTCTTCCTAG TGCCTACAAT His Met Arg Arg 1290	2454
GGGAAACTT CACTCCAAAG AGAAACCTAT TAAGTCATCA TCTCCAACT AAACCCTCAC	2514
AAGTAACAGT TGAAGAAAAA ATGGCAAGAG ATCATATCCT CAGACCAGGT GGAATTACTT	2574

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AAATTTTAAA GCCTGAAAAT TCCAATTTGG GGGTGGGAGG TGGAAAAGGA ACCCAATTTT 2634
CTTATGAACA GATATTTTAA ACTTAATGGC ACAAAGTCTT AGAATATTAT TATGTGCCCC 2694
GTGTTCCCTG TTCTTCGTTG CTGCATTTTC TTCATTGCA GGCAAACTTG GCTCTCAATA 2754
AACTTTTCG 2763

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## (2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 799 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```

Met Val Leu Leu Arg Val Leu Ile Leu Leu Leu Ser Trp Ala Ala Gly
 1      5      10      15
Met Gly Gly Gln Tyr Gly Asn Pro Leu Asn Lys Tyr Ile Arg His Tyr
 20      25      30
Glu Gly Leu Ser Tyr Asn Val Asp Ser Leu His Gln Lys His Gln Arg
 35      40      45
Ala Lys Arg Ala Val Ser His Glu Asp Gln Phe Leu Arg Leu Asp Phe
 50      55      60
His Ala His Gly Arg His Phe Asn Leu Arg Met Lys Arg Asp Thr Ser
 65      70      75      80
Leu Phe Ser Asp Glu Phe Lys Val Glu Thr Ser Asn Lys Val Leu Asp
 85      90      95
Tyr Asp Thr Ser His Ile Tyr Thr Gly His Ile Tyr Gly Glu Glu Gly
100      105      110
Ser Phe Ser His Gly Ser Val Ile Asp Gly Arg Phe Glu Gly Phe Ile
115      120      125
Gln Thr Arg Gly Gly Thr Phe Tyr Val Glu Pro Ala Glu Arg Tyr Ile
130      135      140
Lys Asp Arg Thr Leu Pro Phe His Ser Val Ile Tyr His Glu Asp Asp
145      150      155      160
Ile Ser Glu Arg Leu Lys Leu Arg Leu Arg Lys Leu Met Ser Leu Glu
165      170      175
Leu Trp Thr Ser Cys Cys Leu Pro Cys Ala Leu Leu Leu His Ser Trp
180      185      190
Lys Lys Ala Val Asn Ser His Cys Leu Tyr Phe Lys Asp Phe Trp Gly
195      200      205

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WO 97/31931

PCT/US97/03217

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AAATTTTAAA GCCTGAAAAT TCCAATTTGG GGGTGGGAGG TGGAAAAGGA ACCCAATTTT 2634
CTTATGAACA GATATTTTAA ACTTAATGGC ACAAAGTCTT AGAATATTAT TATGTGCCCC 2694
GTGTTCCCTG TTCTTCGTTG CTGCATTTTC TTCATTGCA GGCAAACTTG GCTCTCAATA 2754
AACTTTTCG 2763

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## (2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 799 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```

Met Val Leu Leu Arg Val Leu Ile Leu Leu Leu Ser Trp Ala Ala Gly
 1      5      10      15
Met Gly Gly Gln Tyr Gly Asn Pro Leu Asn Lys Tyr Ile Arg His Tyr
 20      25      30
Glu Gly Leu Ser Tyr Asn Val Asp Ser Leu His Gln Lys His Gln Arg
 35      40      45
Ala Lys Arg Ala Val Ser His Glu Asp Gln Phe Leu Arg Leu Asp Phe
 50      55      60
His Ala His Gly Arg His Phe Asn Leu Arg Met Lys Arg Asp Thr Ser
 65      70      75      80
Leu Phe Ser Asp Glu Phe Lys Val Glu Thr Ser Asn Lys Val Leu Asp
 85      90      95
Tyr Asp Thr Ser His Ile Tyr Thr Gly His Ile Tyr Gly Glu Glu Gly
100      105      110
Ser Phe Ser His Gly Ser Val Ile Asp Gly Arg Phe Glu Gly Phe Ile
115      120      125
Gln Thr Arg Gly Gly Thr Phe Tyr Val Glu Pro Ala Glu Arg Tyr Ile
130      135      140
Lys Asp Arg Thr Leu Pro Phe His Ser Val Ile Tyr Glu Val Ile
145      150      155

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Phe Ser Glu Ile Tyr Tyr Pro His Lys Tyr Gly Pro Gln Gly Gly Cys  
 210 215 220  
 Ala Asp His Ser Val Phe Glu Arg Met Arg Lys Tyr Gln Met Thr Gly  
 225 230 235 240  
 Val Glu Glu Val Thr Gln Ile Pro Gln Glu Glu His Ala Ala Asn Gly  
 245 250 255  
 Pro Glu Leu Leu Arg Lys Arg Arg Thr Thr Ser Ala Glu Lys Asn Thr  
 260 265 270  
 Cys Gln Leu Tyr Ile Gln Thr Asp His Leu Phe Phe Lys Tyr Tyr Gly  
 275 280 285  
 Thr Arg Glu Ala Val Ile Ala Gln Ile Ser Ser His Val Lys Ala Ile  
 290 295 300  
 Asp Thr Ile Tyr Gln Thr Thr Asp Phe Ser Gly Ile Arg Asn Ile Ser  
 305 310 315 320  
 Phe Met Val Lys Arg Ile Arg Ile Asn Thr Thr Ala Asp Glu Lys Asp  
 325 330 335  
 Pro Thr Asn Pro Phe Arg Phe Pro Asn Ile Ser Val Glu Lys Phe Leu  
 340 345 350  
 Glu Leu Asn Ser Glu Gln Asn His Asp Asp Tyr Cys Leu Ala Tyr Val  
 355 360 365  
 Phe Thr Asp Arg Asp Phe Asp Asp Gly Val Leu Gly Leu Ala Trp Val  
 370 375 380  
 Gly Ala Pro Ser Gly Ser Ser Gly Gly Ile Cys Glu Lys Ser Lys Leu  
 385 390 395 400  
 Tyr Ser Asp Gly Lys Lys Lys Ser Leu Asn Thr Gly Ile Ile Thr Val  
 405 410 415  
 Gln Asn Tyr Gly Ser His Val Pro Pro Lys Val Ser His Ile Thr Phe  
 420 425 430  
 Ala His Glu Val Gly His Asn Phe Gly Ser Pro His Asp Ser Gly Thr  
 435 440 445  
 Glu Cys Thr Pro Gly Glu Ser Lys Asn Leu Gly Gln Lys Glu Asn Gly  
 450 455 460  
 Asn Tyr Ile Met Tyr Ala Arg Ala Thr Ser Gly Asp Lys Leu Asn Asn  
 465 470 475 480  
 Asn Lys Phe Ser Leu Cys Ser Ile Arg Asn Ile Ser Gln Val Leu Glu  
 485 490 495  
 Lys Lys Arg Asn Asn Cys Phe Val Glu Ser Gly Gln Pro Ile Cys Gly  
 500 505 510  
 Asn Gly Met Val Glu Gln Gly Glu Glu Cys Asp Cys Gly Tyr Ser Asp  
 515 520 525

Gln Cys Lys Asp Glu Cys Cys Phe Asp Ala Asn Gln Pro Glu Gly Arg  
 530 535 540  
 Lys Cys Lys Leu Lys Pro Gly Lys Gln Cys Ser Pro Ser Gln Gly Pro  
 545 550 555 560  
 Cys Cys Thr Ala Gln Cys Ala Phe Lys Ser Lys Ser Glu Lys Cys Arg  
 565 570 575  
 Asp Asp Ser Asp Cys Ala Arg Glu Gly Ile Cys Asn Gly Phe Thr Ala  
 580 585 590  
 Leu Cys Pro Ala Ser Asp Pro Lys Pro Asn Phe Thr Asp Cys Asn Arg  
 595 600 605  
 His Thr Gln Val Cys Ile Asn Gly Gln Cys Ala Gly Ser Ile Cys Glu  
 610 615 620  
 Lys Tyr Gly Leu Glu Glu Cys Thr Cys Ala Ser Ser Asp Gly Lys Asp  
 625 630 635 640  
 Asp Lys Glu Leu Cys His Val Cys Cys Met Lys Lys Met Asp Pro Ser  
 645 650 655  
 Thr Cys Ala Ser Thr Gly Ser Val Gln Trp Ser Arg His Phe Ser Gly  
 660 665 670  
 Arg Thr Ile Thr Leu Gln Pro Gly Ser Pro Cys Asn Asp Phe Arg Gly  
 675 680 685  
 Tyr Cys Asp Val Phe Met Arg Cys Arg Leu Val Asp Ala Asp Gly Pro  
 690 695 700  
 Leu Ala Arg Leu Lys Lys Ala Ile Phe Ser Pro Glu Leu Tyr Glu Asn  
 705 710 715 720  
 Ile Ala Glu Trp Ile Val Ala His Trp Trp Ala Val Leu Leu Met Gly  
 725 730 735  
 Ile Ala Leu Ile Met Leu Met Ala Gly Phe Ile Lys Ile Cys Ser Val  
 740 745 750  
 His Thr Pro Ser Ser Asn Pro Lys Leu Pro Pro Pro Lys Pro Leu Pro  
 755 760 765  
 Gly Thr Leu Lys Arg Arg Arg Pro Pro Gln Pro Ile Gln Gln Pro Gln  
 770 775 780  
 Arg Gln Arg Pro Arg Glu Ser Tyr Gln Met Gly His Met Arg Arg  
 785 790 795

**WHAT IS CLAIMED IS:**

1. A DNA fragment encoding a human disintegrin expressed differentially during arthritis development, capable as being used as a screen disintegrin antagonism, drug design and screening.
2. A human disintegrin according to Claim 1 of a molecular weight, and solubility useful as a drug screening agent.
3. A human disintegrin according to Claim 1 in essentially pure form.
4. A screening method for compounds capable of binding to a human disintegrin, comprising the disintegrin of Claim 1.
5. A screening kit for compounds capable of binding to a human disintegrin, comprising the disintegrin of Claim 1.
6. An antibody, or fragment thereof, to human disintegrin of Claim 1.
7. A screening method for a metalloprotease mediated disease comprising the administration of an antibody according to Claim 6 and observing its effect.
8. A screening method for osteoarthritis comprising the administration of an antibody according to Claim 7 and observing its effect.
9. A screening method for osteoarthritis according to Claim 7, where blood, synovial fluid or other body fluids are screened.
10. A screening kit for osteoarthritis comprising an antibody, or fragment thereof, to human disintegrin of Claim 6.
11. A screening method, according to Claim 4, useful in determining relative potency in treating osteoarthritis.

12. DNA encoding the disintegrin of Claim 1 (Seq ID NO 2).
13. An expression vector or plasmid comprising the DNA of Claim 12.
14. A cell comprising the DNA of Claim 12.
15. A cell comprising the expression vector or plasmid of Claim 13.
16. The cell of Claim 14 where the DNA is foreign to that cell.
17. An inhibitor of the human disintegrin of Claim 2.
18. A method of treating a disease state associated with disintegrin activity.
19. The disintegrin of Claim 2, wherein the disintegrin is aggrocane.
20. A method of treating a disease state according to Claim 18 wherein the disease is an arthropathy.
21. A method according to Claim 20, wherein the disease is osteoarthritis.
22. The disintegrin of Claim 2, wherein the disintegrin modulates tissue remodeling or breakdown.

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US97/03217

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(6) : Please See Extra Sheet.

US CL : 536/23.5; 435/7.1, 320.1, 325; 424/130.1; 514/2; 530/350;

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/23.5, 23.1; 435/7.1, 4, 320.1, 325; 424/130.1; 514/2; 530/350

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
Please See Extra Sheet.**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Database Genbank on STN, Institute of Electrical Engineers, (Stevenage, GB, USA), Genbank ACC. No. D31872, KATAGIRI et al., 'Human metalloprotease/disintegrin-like (MDC) gene: exon-intron organization and alternative splicing,' sequence, Cytogenet. Cell Genet., 68 (1-2), 39-44, 1995, first submitted 18 June 1994, see entire sequence listing.	1-22
X	Database Genbank on STN, Institute of Electrical Engineers, (Stevenage, GB, USA), Genbank ACC. No. U41767, BLOBEL, C. P., DIRECT SUBMISSION, sequence submitted 04 December 1995, see entire sequence listing.	1
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Y		2-5

☒ Further documents are listed in the continuation of Box C.
 ☐ See patent family annex.

*A*	document defining the general state of the art which is not considered to be of particular relevance	*T*	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*E*	earlier document published on or after the international filing date	*X*	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*L*	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Y*	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*O*	document referring to an oral disclosure, use, exhibition or other means	*Z*	document member of the same patent family
*P*	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

17 APRIL 1997

Date of mailing of the international search report

09 JUL 1997

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## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US97/03217

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	Database Genbank on STN, Institute of Electrical Engineers, (Stevenage, GB, USA), Genbank ACC. No. U41766, BLOBEL C. P., DIRECT SUBMISSION, sequence submitted 04 December 1995, see entire sequence listing.	1 ----- 2-5
X, P --- Y, P	KRATZSCHMAR et al. Metargidin, a membrane-anchored metalloprotease-disintegrin protein with an RGD Integrin binding sequence. J. of Biol. Chem. 01 March 1996, Vol. 271, No. 9, pages 4593-4596, especially pages 4593-4594.	1, 4, 6 ----- 2, 3, 5, 7
X --- Y	WESKAMP et al. MDC9, a widely expressed cellular disintegrin containing cytoplasmic SH3 ligand domains. J. of Cell Biol. 04 February 1996, Vol. 132, No. 4, pages 717-726, especially pages 717-718.	1, 4, 6 ----- 2, 3, 5, 7

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US97/03217

## A. CLASSIFICATION OF SUBJECT MATTER: IPC (6):

C07H 21/02; G01N 33/53; C12N 15/63, 5/16; A61K 39/395, 38/00; C07K 14/435

## B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

APS, Medline, Scisearch, Biosis, Embase, CAPLus, WPIDS, Genbank

Search terms: disintegrin, DNA, cDNA, coding sequence, screen, bind, metalloprotease, osteoarthritis, aggrecanase, arthritis, human, Haqqi, T/au, Tindal, M/au